Stage Drift Characterisation

Introduction

In order to perform time-lapse imaging, the digital microscope must be left capturing images for long periods of time. However, most microscope stages exhibit 'drift', which causes the sample to move during the experiment. This can seriously affect the results, and must be fully characterised before solutions are developed. The causes of drift are complex, and can be as a result of thermal expansion, mechanical movement and vibrations amongst other factors [1] [2].

In order to characterise our prototype brightfield microscope, an experiment was set-up to record the movement of points (fiducial mark) on a slide over a period of roughly 16 hours. This is an established technique for measuring drift [3]. A free, open-source video analysis and modelling software was used to manually track the points over time. Two key conditions were tested: the first was the normal working set-up, the second was carried out with the screws linking the translation knobs to the stage itself removed. This essentially uncouples the stage from the translation knobs, and the sample is then moved until it's in the correct orientation. The experimental objective was to characterise the movements of our 3D printed stage in isolation, and compare this to its movements when coupled to the translation knobs as it would be when fully functional.

Brightfield Test 1

Methods

- Time lapse set up:
  - Raspberry Pi camera set up to take images at 5 min intervals from 16.45pm to 09.30am
  - Used prototype brightfield microscope, with a standard micrometer slide
  - Code used is summarised in https://www.raspberrypi.org/learning/timelapse-setup/
- Movie creation and tracking:
  - Used iMovie to compile still images into a single movie. Captured 203 images, taken every 5 minutes, displayed for 0.4s each
  - A single point was tracked using Tracker (http://physlets.org/tracker/). Tracking was carried out over intervals of 20 frames across the entire time lapse
  - Calibration was carried out using the micrometer scale
  - Discounted final image (No. 203), as this was taken after the slide have been moved slightly when we returned to the lab to check the results
  - Image tracking was repeated 4 times, and each time the x and y coordinates were recorded. This was an attempt to increase the reliability of the results
  - The data were combined, and the average plotted with error bars indicating 1 SD

  Note: tracking involves selecting the target point by eye, which may introduce a significant amount of error

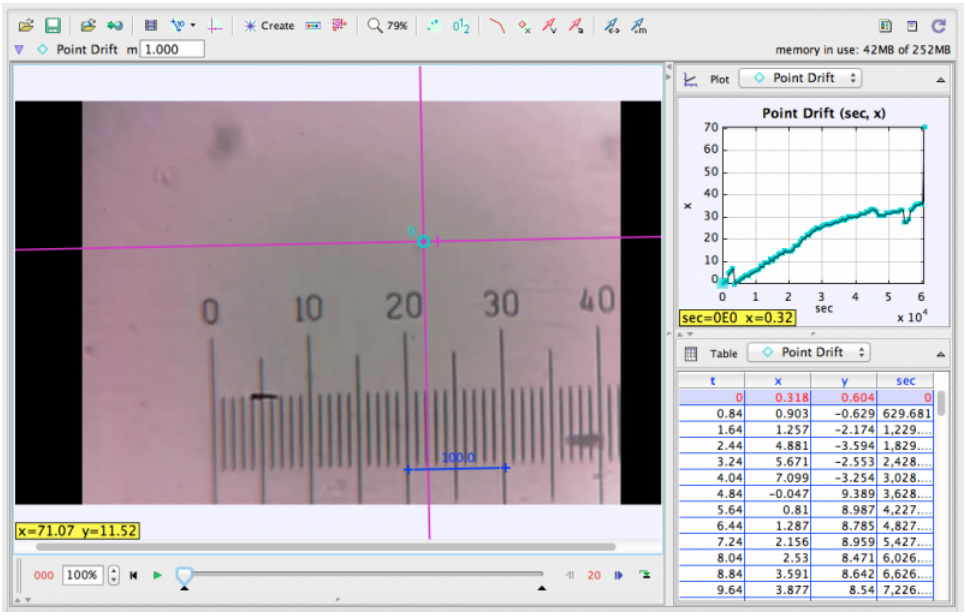

Figure 1: *Scale bar (dark blue) was used for calibration. Origin was initially centered on the point being tracked. Point being tracked is marked at t=0 (light blue circle). Axis and calibration bar were aligned with the micrometer scale by eye*

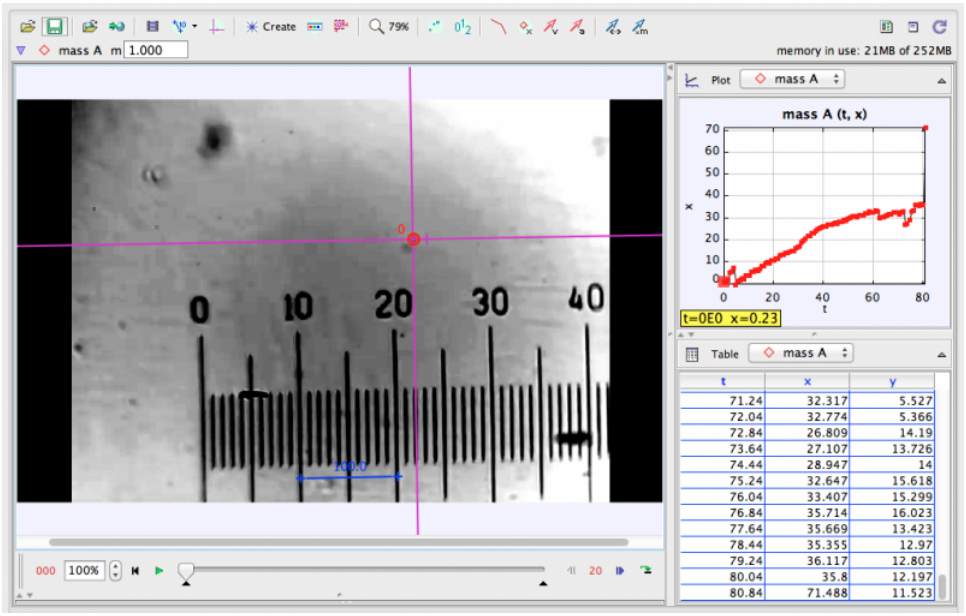

Figure 2: *In order to increase the accuracy of the tracking, a filter was used to convert the image to grayscale and the contrast was increased.*

Results

- Average movement speed along x-axis = 0.0006 μm/s (across entire timelapse)
- Average movement speed along y-axis = 0.0002 μm/s (across entire timelapse)

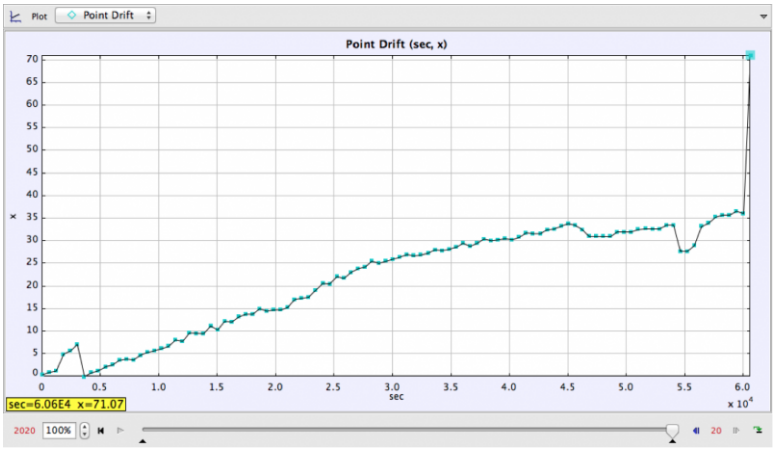

Figure 3: *A single point on the slide was tracked at intervals of 20 frames for the entire length of the time lapse (60,600s). Time (s) is plotted on the horizontal, with distance from origin (μm) along x-axis plotted on the vertical axis.*

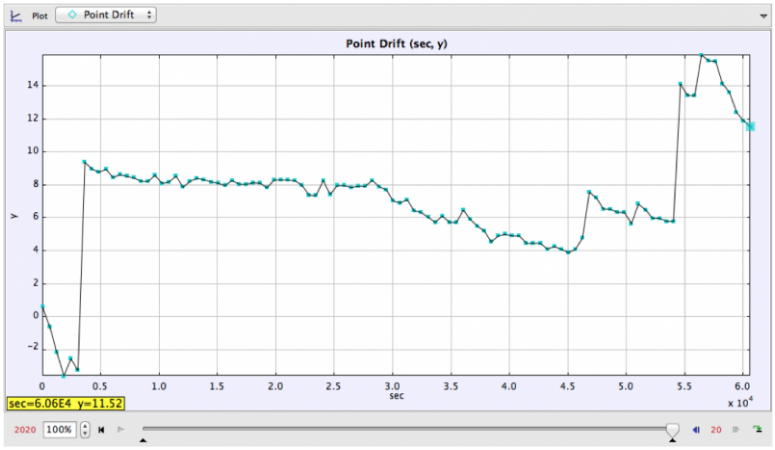

Figure 4: *A single point on the slide was tracked at intervals of 20 frames for the entire length of the time lapse (60,600s). Time (s) is plotted on the horizontal, with distance from origin (μm) along y-axis plotted on the vertical axis.*

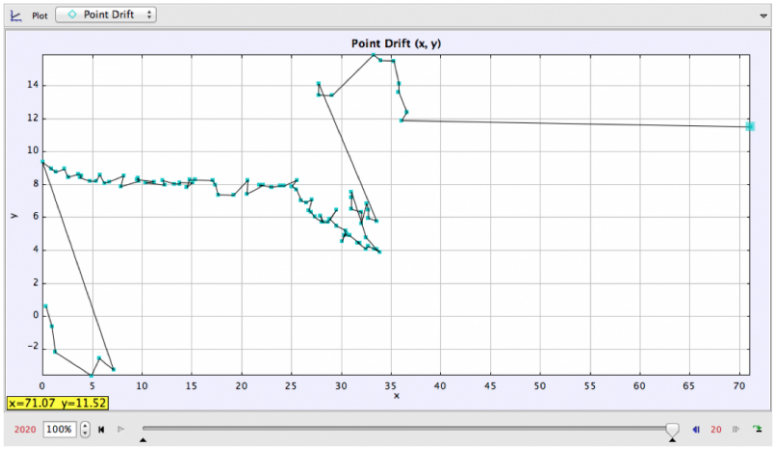

Figure 5: *A single point on the slide was tracked at intervals of 20 frames for the entire length of the time lapse (60,600s). Distance from origin (μm) along x-axis is plotted on the horizontal, and distance from origin (μm) along y-axis is plotted on the vertical.*

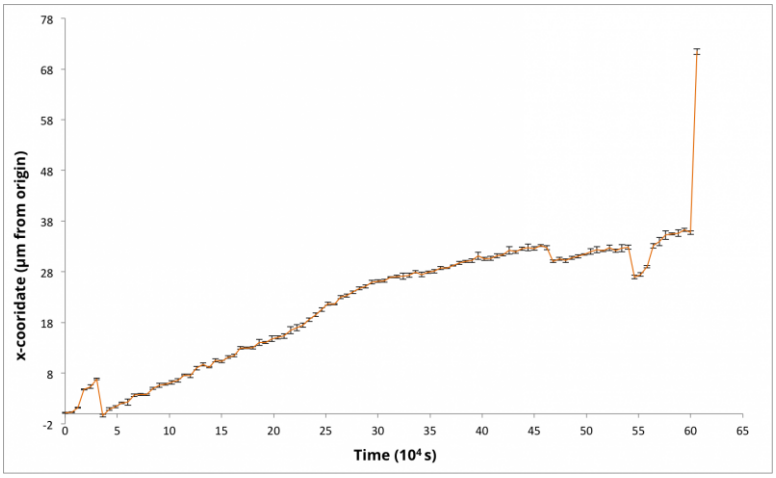

Figure 6: *: Graph shows aggregated data from 4 trials in BT1. In all trials, the axis and calibration scale were consistent. Error bars indicate 1 SD.*

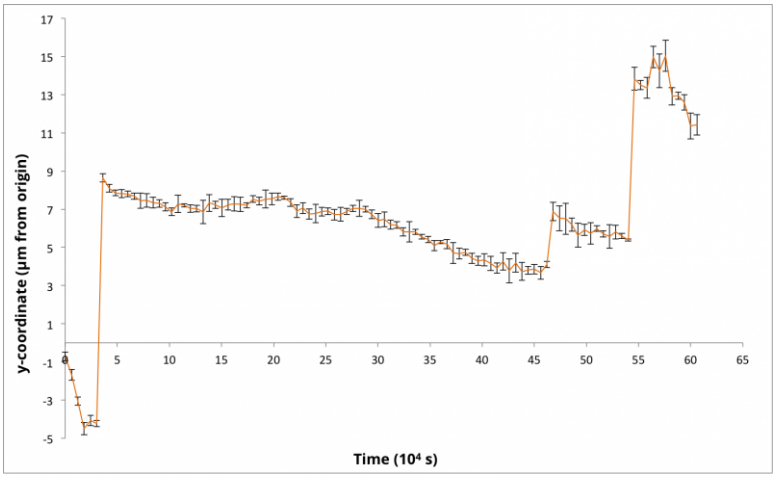

Figure 7: *Graph shows aggregated data from 4 trials in BT1. In all trials, the axis and calibration scale were consistent. Error bars indicate 1 SD.*

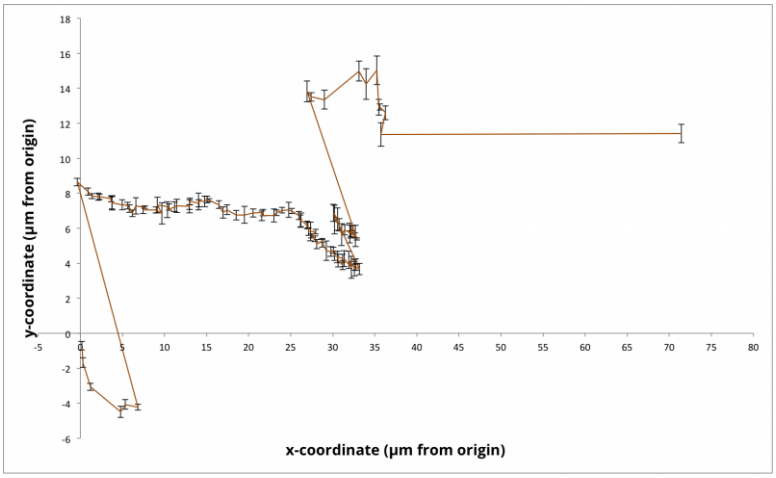

Figure 8: *Graph shows aggregated data from 4 trials in BT1. In all trials, the axis and calibration scale were consistent. Error bars indicate 1 SD.*

Brightfield Test 2

Methods

- Time lapse set up:
  - Raspberry Pi camera set up to take images at 5 min intervals over a period of approximately 16 hours
  - Used prototype brightfield microscope, with a standard micrometer slide
  - Screws connecting the chassis to the manual controllers were removed, leaving the stage itself in isolation
  - Code used is summarised in https://www.raspberrypi.org/learning/timelapse-setup/
- Movie creation and tracking:
  - Used iMovie to compile still images into a single movie. Captured 200 images, taken every 5 minutes, displayed for 0.4s each
  - A single point was tracked using Tracker (http://physlets.org/tracker/). Tracking was carried out over intervals of 20 frames across the entire time lapse
  - Calibration was carried out using the micrometer scale. Note that the ooint tracked in BT2 was not the same as thet tracked in BT1.
  - Image tracking was repeated 4 times, and each time the x and y coordinates were recorded. This was an attempt to increase the reliability of the results by reducing the effect of human error
  - The data were combined, and the average plotted with error bars indicating 1 SD
- Comparison of tests 1 and 2:
  - Translated results from Test 2 such that both start at the same origin (assume that initial points are effectively the same)
  - Consider the movement of the starting point under two different conditions: Screws and No Screws
  - Plot coordinates on same graph to give an informative comparison of the two movements

  Note: Error bars all 1 SD. With screws: 60,600 seconds (202 points). Without screws: 60,000 seconds (200 points)

Results

- Average movement speed along x-axis = 0.0031 μm/s (across entire timelapse)
- Average movement speed along y-axis = 0.0025 μm/s (across entire timelapse)

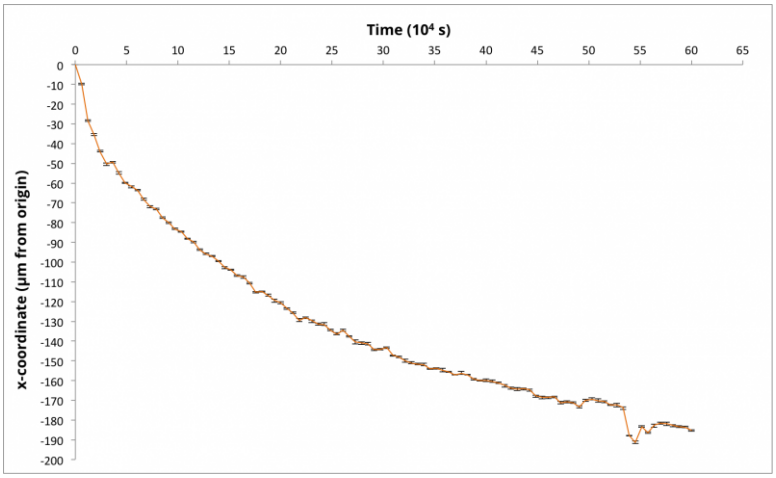

Figure 9: *Graph shows aggregated data from 4 trials in BT2. In all trials, the axis and calibration scale were consistent. Error bars indicate 1 SD.*

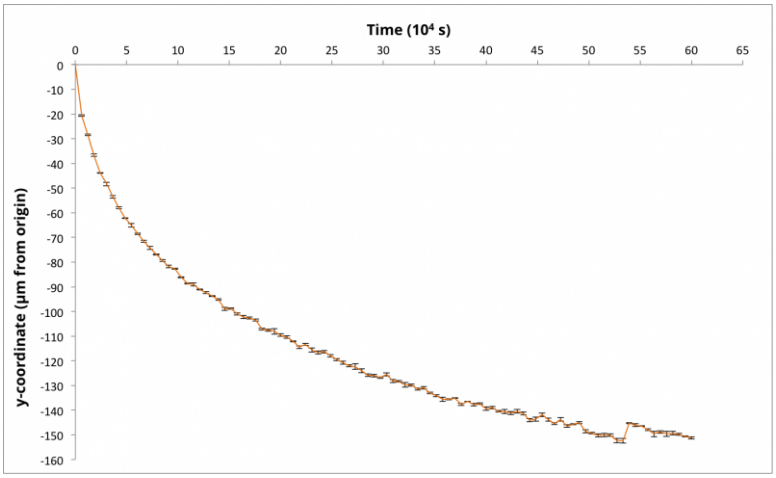

Figure 10: *Graph shows aggregated data from 4 trials in BT2. In all trials, the axis and calibration scale were consistent. Error bars indicate 1 SD.*

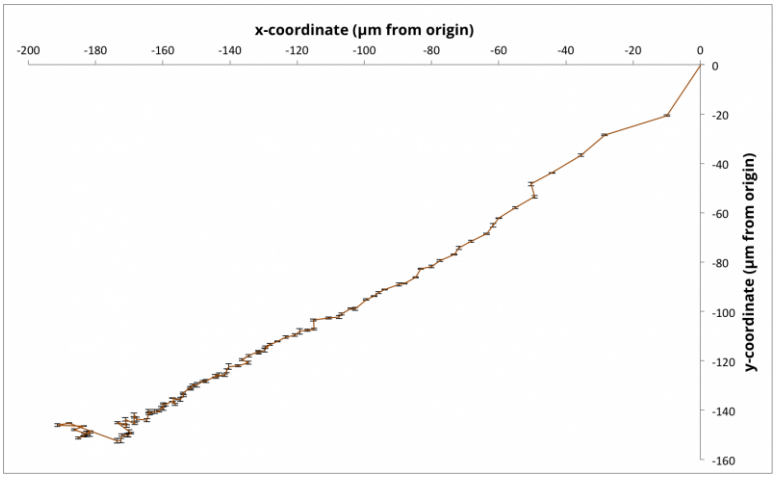

Figure 11: *Graph shows aggregated data from 4 trials in BT2. In all trials, the axis and calibration scale were consistent. Error bars indicate 1 SD.*

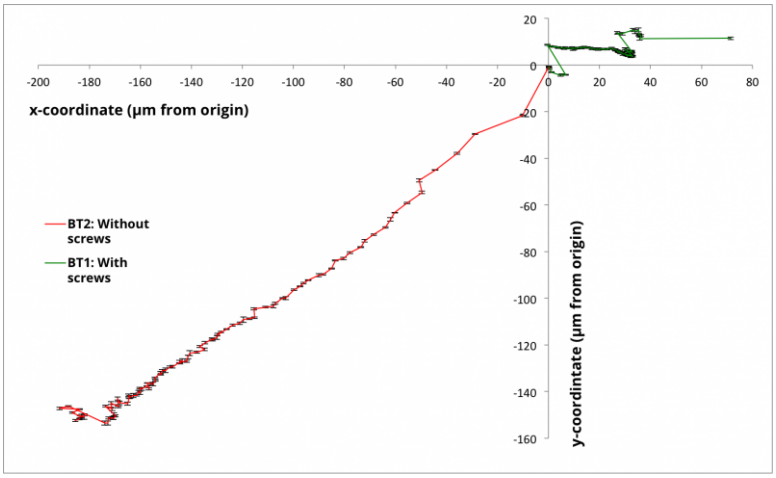

Figure 12: *Graph shows aggregated data from 4 trials in BT1 and in BT2 normalised of the same start point. Assumes points experience the same drift anywhere on the slide. In all trials, the axes and calibration scale were consistent. They were not consistent between BT1 and BT2. Error bars indicate 1 SD.*

Discussion

Results indicate that the attachment of the screws significantly reduces microscope drift compared to the stage with screws unattached, but drift is still significant in both cases and will pose a problem for attempts to carry out accurate time-lapse imaging. A qualitative analysis of the results from BT2 suggest from the behaviour of the drift that the stage gradually approaches a stable equilibrium position. One potential solution may therefore be to determine the time taken to reach the equilibrium, and to image only once this has occurred. This will require further experimentation to determine whether the stage does actually reach a stable equilibrium, and how long this takes.

However, with a fully motorised stage the screws must be attached, which changes the dynamics of the drift dramatically and discounts this option. Solutions have been proposed that make use of software to counteract drift using autofocus (for drift along z-axis). Additionally piezoelectric stages and automated stepper motors to actively remove drift (for movement along x-axis and y-axis) have also been implemented with commercial microscopes[3] [4]. In current fluorescent systems artificially labelled beads can be used as fiducial marks in the sample plane. Using a closed-loop feedback system, stage drift can then be corrected for based on the position of the fiduciary mark relative to the camera sensor [2]. Many of these techniques are too technically complex to implement for our microscope, and beyond the scope of our capabilities. However the implementation of active drift correction using stepper motors is being considered. This must be integrated with image recognition software to detect movement away from the sample being imaged.

Further testing, under different conditions such as different temperatures, will allow determination of the cause of drift when the screws are attached. However, this is likely to be due to a number of factors each making a small contribution to overall stage movement.

References

[1] Microscopyu.com, (2015). Nikon MicroscopyU — Live-Cell Imaging — Focus Drift Correction. [online] Available at: https://www.microscopyu.com/articles/livecellimaging/focusdrift.html [Accessed 10 Aug. 2015].

[2] Microscopedrift.com, (2015). Microscope Drift — The Solution to Focus Drift and Sample Drift. [online]

Available at:
http://microscopedrift.com/ [Accessed 10 Aug. 2015].

[3] Carter, A., King, G., Ulrich, T., Halsey, W., Alchenberger, D. and Perkins, T. (2007). Stabilization of an optical microscope to 0.1 nm in three dimensions. Appl. Opt., 46(3), p.421.

[4] Kreft, M., Stenovec, M. and Zorec, R. (2005). Focus-Drift Correction in Time-Lapse Confocal Imaging. Annals of the New York Academy of Sciences, 1048(1), pp.321-330.